Granzyme B

Concentrated and Prediluted Monoclonal Antibody
901-3202-090817

BIOCARE MEDICAL

Catalog Number:	**ACI 3202 A, B**	**API 3202 AA**
Description:	0.1, 0.5 ml, concentrate	6.0 ml, prediluted
Dilution:	1:50	Ready-to-use
Diluent:	Da Vinci Green	N/A

Intended Use:

For In Vitro Diagnostic Use

Granzyme B [11F1] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of granzyme B protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

Cytotoxic lymphocytes, including NK cells and cytotoxic T lymphocytes (CTLs), play a major role in the defense against neoplastic processes and viral infections (1). Granule exocytosis is the mechanism by which cytotoxic lymphocytes may induce lysis of its target. This involves granule-associated cytotoxic proteins, including the T-cell intracellular antigen-1 (TIA-1), perforin and granzyme B (1,2). Perforin produces pores that allow the entry of other cytotoxic proteins such as TIA-1 and granzymes, which trigger a process leading to DNA fragmentation and apoptosis of the target cells (1). However, there is evidence that granzyme B may kill targets independently of perforin (2). Recent studies (3,4,5) have demonstrated that the expression of granzyme B by immunohistochemistry in several entities of extranodal peripheral T cell lymphoma (PTCL) and NK cell lymphomas, including nasal and nasal-type NK/T cell lymphomas, hepatosplenic and non-hepatosplenic PTCL, enteropathy-type (ETCL) and non-ETCL intestinal PTCL, subcutaneous panniculitis-like PTCL (SPTCL), cutaneous CD8+ epidermotropic lymphomas as well as in nodal and cutaneous CD30+ anaplastic large cell lymphomas (ALCL) (3,4,5). In contrast, only a few nodal PTCL-unspecified (UC) express cytotoxic phenotype while angioimmunoblastic (AILD) lymphomas do not (3,4,5). The expression of cytotoxic proteins, such as granzyme B may be important for the identification and classification of extranodal T- and NK-cell lymphomas since many of these tumors do not have specific morphology and phenotype (6,7).

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 11F1

Isotype: IgG2a

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Recombinant protein corresponding to the N-terminus of granzyme B

Cellular Localization: Cytoplasmic lytic granules

Positive Tissue Control: Spleen or tonsil

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations:

Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.

Pretreatment: Perform heat retrieval using Biocare's Borg or Diva Decloaker. Refer to the Borg or Diva Decloaker product data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Notes:

1. This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.
2. Use Borg Decloaker for lymphoid tissue. Use Diva Decloaker for prostate or breast. Does not stain with Reveal Decloaker.

Performance Characteristics:

Sensitivity and specificity on diseased tissue and tissue cross-reactivity on normal tissue is summarized in Tables 1 and 2, respectively.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if

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Precautions Cont'd:
ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (8)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (9)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:
1. Smyth MJ, Trapani JA. Granzymes: exogenous proteinases that induce target cell apoptosis. Immunol Today. 1995 Apr;16(4):202-6.
2. Froelich CJ, Dixit VM, Yang X. Lymphocyte granule-mediated apoptosis: matters of viral mimicry and deadly proteases. Immunol Today. 1998 Jan;19(1):30-6.
3. Krenacs L, *et al*. Cytotoxic cell antigen expression in anaplastic large cell lymphomas of T- and null-cell type and Hodgkin's disease: evidence for distinct cellular origin. Blood. 1997 Feb 1;89(3):980-9.
4. Kanavaros P, *et al*. Cytotoxic protein expression in non-Hodgkin's lymphoma and Hodgkin's disease. Anticancer Res. 1999 Mar-Apr;19(2A):1209-16.
5. Kanavaros P, *et al*. Expression of cytotoxic proteins in peripheral T-cell and natural killer-cell (NK) lymphomas: association with extranodal site, NK or Tgammadelta phenotype, anaplastic morphology and CD30 expression. Leuk Lymphoma. 2000 Jul;38(3-4):317-26.
6. Jaffe ES, *et al*. Extranodal peripheral T-cell and NK-cell neoplasms. Am J Clin Pathol. 1999 Jan;111(1 Suppl 1):S46-55. Review.
7. Kinney MC. The role of morphologic features, phenotype, genotype and anatomic site in defining extranodal T-cell and NK-cell neoplasms. Am J Clin Pathol. 1999 Jan;111(1 Suppl 1):S104-18.
8. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
9. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Table 1: Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Breast Cancer	0	9
Colon Cancer	0	18
Lung Cancer	0	30
Prostate Cancer	0	14
Renal Cancer	0	20
Bladder Cancer	0	12
Melanoma	0	5
Ovarian Cancer	0	9
Peripheral T Cell Lymphoma	1	1

Note: Tumor infiltrating-lymphocytes are positive.

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	0	3
Cerebellum	0	3
Adrenal	0	3
Ovary	0	2
Pancreas	0	3
Parathyroid	0	2
Pituitary	0	3
Testis	0	3
Thyroid	0	3
Breast	0	3
Spleen	3	3
Tonsil	3	3
Thymus	0	3
Bone Marrow	0	3
Lung	0	3
Heart	0	3
Esophagus	0	1
Small Intestine	0	3
Colon	0	3
Liver	0	3
Salivary Gland	0	3
Kidney	0	3
Prostate	0	3
Uterus	0	2
Cervix	0	3
Skeletal Muscle	0	3
Skin	0	3
Peripheral Nerve	0	3
Linging Cells	0	3

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